Amendments to the Drawing:

Beginning on pages 3-6, please amend the description of the drawings as shown below. No new matter has been added.

Figures 1A and 1B (not shown):

BNP expression after induction of SHOX

Figure 1A: Semiquantitative RT-PCR with SHOX or BNP specific primers was performed on total RNA isolated from U2OS-SHOX or U2OS-STM cells 48 hours after induction (ind) of protein expression and on RNA from uninduced control cells (unind). BNP is detectable only upon induction of the full length SHOX protein in the induced U2OS-SHOX cells.

Figure 1B: (not shown) Total RNA was extracted from the inducible cell line U2OS-SHOX which expressed SHOX at 0, 12, 24, 36, 48 and 72 hours or from uninduced U2OS control cells. Concentration of BNP mRNA was determined by quantitative RT-PCR carried out in duplicate using GAPDH as a standard. BNP mRNA levels (diamonds) increased significantly with time compared to the uninduced cells (squares).

Figures 2A and 2B:

Electromobility Shift Assay (EMSA) of the proximal SHOX binding site BNP-600.

Figure 2A: 10 fmol of ^{32}P-radiolabelled double-stranded oligonucleotide containing the putative proximal binding site of SHOX was incubated with 0, 0.05, 0.5 and 3 µl purified SHOX-GST (250 nM). Monomeric binding of SHOX-GST could be observed with volumes of 0.05 and 0.5 µl, an increase in SHOX-GST concentration led to the formation of homeodimers.

Competition: Incubation of 1 µl of SHOX-GST with 10 fmol radiolabelled oligonucleotide and an increasing (0, 50 x, 150 x, 500 x and 1000 x) excess of unlabelled oligonucleotide resulted in a decrease in signal intensity.

Supershift: anti-SHOX antibody (AB) was added to the oligonucleotide-SHOX-GST complex. An additional shift of the monomeric SHOX-GST-Oligonucleotide complex

could be observed, which had not been seen in the controls, indicating the binding of the AB.

M: monomeric binding; D: dimeric binding; SS: supershift. GST: purified GST-tag alone; -: no protein extract added.

Figure 2B: Sequence specificity of the binding.

To test the sequence specificity of the SHOX DNA binding SHOX-GST was incubated with oligonucleotides containing artificially introduced mutations in the putative SHOX binding site. Nucleotides differing from the wild type sequence (Wt) are highlighted in green (BNP-600a, BNP-600b). As the number of mutated nucleotides increased, binding was strongly reduced (BNP-600a) or completely disappeared (BNP-600b).

Figures 3A and 3B:

Electromobility Shift Assay (EMSA) of the distal SHOX binding site BNP-1220.

Figure 3A: 10 fmol of ^{32}P-radiolabelled double stranded oligonucleotide containing the putative distal binding site of SHOX was incubated with 0, 0.05, 0.5 and 3 µl purified SHOX-GST (250 nM). Monomeric binding of SHOX-GST could be observed with volumes of 0.05 and 0.5 µl, an increase in SHOX-GST concentration led to the formation of homeodimers.

Competition: Incubation of 1 µl of STM with 10 fmol radiolabelled oligonucleotide and an increasing (0, 50 x, 150 x and 1000 x) excess of unlabelled oligonucleotide resulted in a decrease in signal intensity.

Supershift: anti-SHOX antibody (AB) was added to the oligonucleotide-SHOX-GST complex. An additional shift of the monomeric SHOX-GST-Oligonucleotide complex could be observed, which was not seen in the controls, indicating the binding of the AB.

M: monomeric binding; D: dimeric binding; SS: supershift. GST: purified GST-tag alone; -: no protein extract added.

Figure 3B: Sequence specificity of the binding.

To test the sequence specificity of the SHOX DNA binding, SHOX-GST was incubated with oligonucleotides containing artificially introduced mutations in the

putative SHOX binding site. Nucleotides differing from the wild-type sequence (Wt) are highlighted in green (BNP-1220a, BNP-1220b, BNP-1220c). As the number of mutated nucleotides increased, binding was strongly reduced (BNP-1220a, BNP-1220b) or completely disappeared (BNP-1220c).

Figures 4A, 4B and 4C:

Figure 4A: Genomic locus of BNP. Exons are represented by blue boxes, start and stop codon are indicated. Sequences of the putative SHOX binding sites (BNP-1220 and BNP-600) are shown.

Figure 4B: Reporter constructs for the activity analysis of the BNP regulatory region. Putative SHOX binding sites in the regulatory region of BNP are indicated. The regulatory region was inserted in forward (BNP for) and reverse (BNP rev) orientation. The construct p3XG was generated by insertion of an experimentally determined SHOX binding site in front of the SV40 core promoter (Rao et al., 2000).

Figure 4C: Luciferase activity after induction of SHOX. Reporter constructs were transiently transfected into U2Os-SHOX or U2OS-STM cells and luciferase activity was determined after 48 hours of SHOX or SHOX-STM induction. With BNP for a 10-fold increase was observed upon induction compared to uninduced control cells. BNP rev revealed an 8-fold and p3XG a 2-fold increase of luciferase activity. No significant changes in luciferase activity were obtained for the negative control vector pGL3promoter. All experiments were performed in triplicate. The bars represent the mean values of two independent experiments.